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Review

Coordinated cell behaviours in early urogenital system morphogenesis

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ABSTRACT

The elaboration of functional kidneys during embryonic development proceeds in a stepwise manner, starting with the formation of the embryonic pro- and mesonephros, followed by the induction and growth of the final metanephric kidney. These early stages of urinary tract development are critical for the embryo as a failure in pro/mesonephros morphogenesis leads to major developmental defects, often incompatible with life. The formation of the pro/mesonephros and its central component the nephric duct, is also interesting as it offers a relatively simple system to study cell biological behaviours underlying tissue morphogenesis. This system is especially well adapted to study the questions of cell lineage specification, epithelial integrity and plasticity, tissue interactions, collective cell migration/guidance and programmed cell death. In this review, we establish the link between these cell behaviours, their molecular regulators and early genitourinary tract development.

Contents

1. Introduction

The study of mammalian urogenital system development has focused mainly on the importance of metanephric (adult) kidney formation. Arguably, however, the earlier events of nephric duct specification and formation are as important for urinary system morphogenesis. The entire renal lineage is derived from the intermediate mesoderm, which epithelializes bilaterally at the level of the 6th to 8th somites to generate the nephric ducts [1]. These paired ducts elongate caudally along the anterior–posterior axis until they reach and fuse with the cloaca, which serves as the precursor of the adult bladder and urethra. During its development the nephric duct gives rise to three distinct renal structures, the pronephros, mesonephros and metanephric kidney, by induction of nephric cord mesoderm adjacent to the nephric duct [2]. Only the metanephric kidney is maintained to adulthood in vertebrates, whereas the pro/mesonephros become part of the genital system in males (vas deferens and epididymis), and degenerate in females.

The metanephric kidney arises from the posterior nephric duct through reciprocal inductive signalling between the metanephric mesenchyme and the nephric duct, causing a budding of the duct known as the ureteric bud. The ureteric bud invades the metanephric mesenchyme, whereupon iterative cycles of branching and mesenchymal-to-epithelial differentiation will give rise to the entire glomerular-nephron-collecting duct system of the adult kidney (reviewed in [3] and Blake and Rosenblum this issue).

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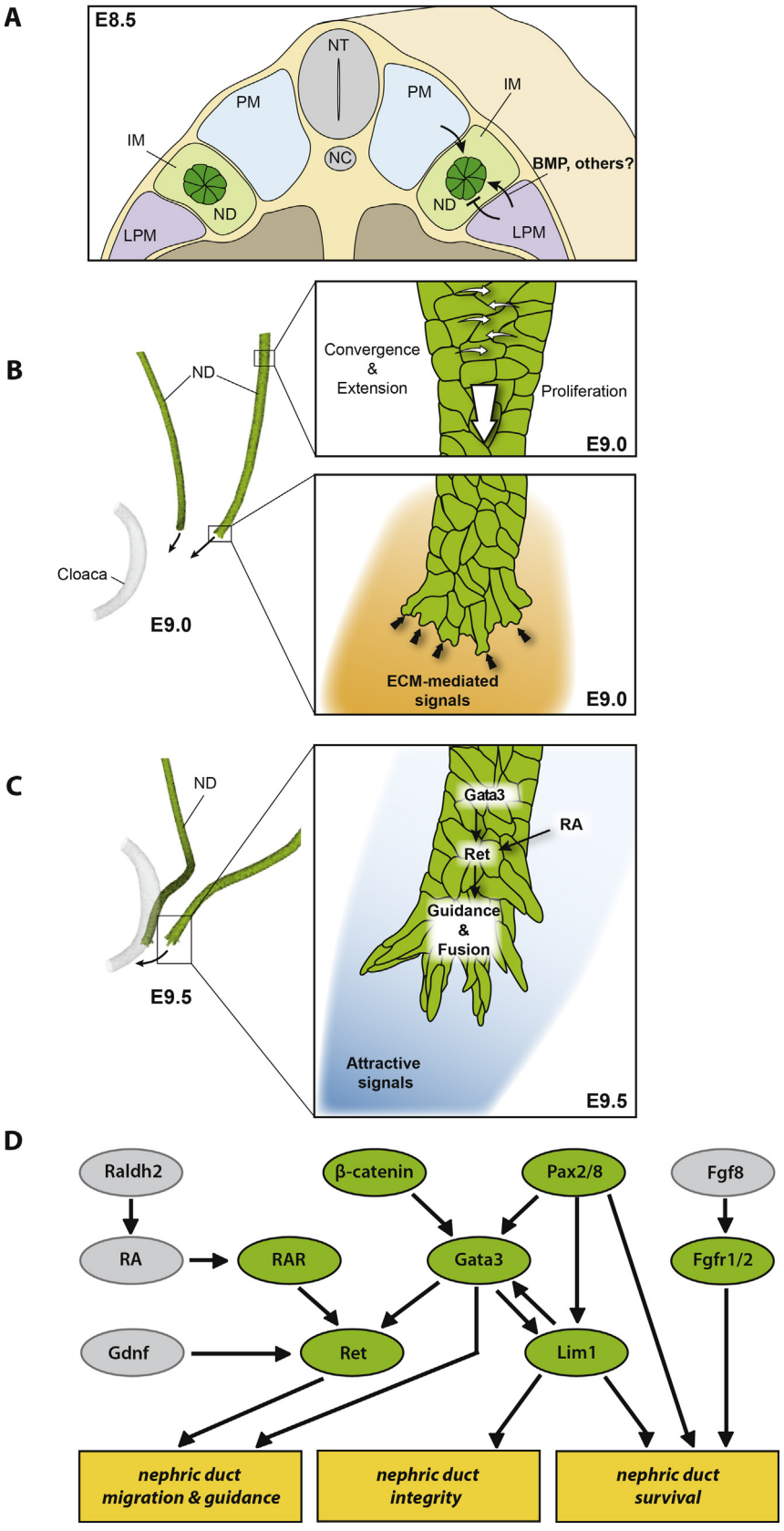

Fig. 1. Early urogenital system development involves coordinated signalling between mesenchymal and epithelial tissues to form and elongate the nephric ducts. (A) Delineation of the renal field is achieved by positive and negative signals emanating from the paraxial mesoderm and lateral plate mesoderm. Bmp signalling from the lateral plate mesoderm and unknown positive signals from the paraxial mesoderm interact to specify the renal field, while negative signals from the lateral plate mesoderm are thought to repress the renal fate laterally. (B) Initial elongation of the nephric duct in amphibian and avian systems involves convergence and extension cell movements (white arrows), as well as proliferation, to drive extension towards the cloaca. Early guidance cues are provided via lamellipodia-like cellular extensions (black arrows) that sense the underlying extracellular matrix. (C) Later guidance cues from the cloacal region are interpreted by the Ret receptor tyrosine kinase activated and maintained by both Gata3

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Concurrently, the segment of ureteric bud that remains outside the metanephric mesenchyme will elongate and differentiate to form the ureter (reviewed in [3] and Bohnenpoll and Kispert this issue). Maturation of the ureter to its final position in the bladder wall requires the apoptotic elimination of a segment of the nephric duct located between the ureteric bud and cloaca (the common nephric duct; CND), thereby allowing the separation of the urinary tracts from the genital tracts [4–6].

Urogenital system morphogenesis requires the coordinated interaction between multiple tissue compartments, primarily along the anteroposterior axis, to appropriately specify the nephric duct primordium, coordinate its differentiation, and guide its elongation in a spatiotemporally defined manner. In this review, we will cover the main aspects of nephric duct morphogenesis and ureter maturation, with a special emphasis on the underlying cell biological mechanisms at play.

2. Renal cell fate specification

The entire urogenital system originates from the intermediate mesoderm in an anterior–posterior continuum, and thus receives patterning signals to appropriately position each region of the kidney field. The anterior intermediate mesoderm is derived from a population of cells that migrate from the mid-primitive streak [7–10]. Commitment to intermediate mesoderm competent for kidney development appears to be at least partially determined prior to cell migration from the primitive streak, as cells from the more posterior primitive streak are unable to contribute to nephric duct formation when grafted into the normal duct forming region [7,8]. Conversely, transplantation of prospective duct forming intermediate mesoderm from the mid-primitive streak to more posterior locations results in nephric duct formation, suggesting that the ability to respond to duct-promoting signals is determined in the primitive streak, and that duct-promoting signals exist throughout the length of the kidney field [7,8]. Tissue recombination experiments, conducted primarily in avian systems, have identified the paraxial and lateral plate mesoderm as the source of signals positioning and specifying the renal primordium (Fig. 1A). Removal of the paraxial mesoderm prior to the 4-somite stage (HH8 in chick embryos) results in the loss of intermediate mesoderm-specific markers and an absence of renal lineage, while the same manipulation at later stages does not appear to affect formation of the pronephros [9]. Interestingly, the paraxial mesoderm signals alone are not sufficient to specify the renal field [9,11], suggesting that an interplay of mediolateral signalling modulates renal field specification (Fig. 1A). The identity of the signals emanating from the paraxial mesoderm region is still elusive.

The role of the lateral plate mesoderm, on the other hand, is better defined. Prior to the 6-somite stage, the lateral plate mesoderm is required as a source of both positive and negative regulatory signals to demarcate the renal field [9,10]. Whereas the inhibitory signals have not been definitively identified, intermediate mesoderm specification appears to be positively regulated by bone morphogenetic protein (BMP) signalling (Fig. 1A) [10]. Remarkably, the requirement for BMP signalling in the intermediate mesoderm is translation-dependent, suggesting the existence of additional factors that directly regulate renal field specification [10]. Additionally, BMP signalling has been implicated in the maintenance of pronephric duct gene expression, including that of *Lim1* (*Lhx1*) and *Pax2*, thereby promoting the survival and differentiation of the nephric duct [12].

In the mouse, the earliest known renal lineage markers are the transcription factors Pax2 and Pax8, which together are both necessary and sufficient to drive nephric duct morphogenesis [1]. Expression of *Pax* genes appears important for nephric duct formation and differentiation, as complete loss of both *Pax2* and *Pax8* results in the absence of the nephric duct, despite normal intermediate mesoderm formation, which can be rescued by retention of one allele of either *Pax* gene [1]. Thus, part of the transcriptional programme driven by Pax2/8 appears to regulate mesenchymal to epithelial transition of nephric duct progenitors, similar to the later requirement for *Pax2* in nephron induction in the metanephric kidney [13]. Interestingly, misexpression of Pax2 in the mesoderm is sufficient to induce an ectopic nephric duct [1], though again, mostly in a region normally competent for duct formation, suggesting that Pax2 cooperates with additional factors in the intermediate mesoderm to drive mesenchymal-epithelial transition and renal fate specification.

3. Epithelial duct integrity and cell survival

Recent work has placed Pax2 and Pax8 at the onset of a gene regulatory network that regulates pro/mesonephros development in a cell-autonomous fashion ([14]; reviewed in [15]). Downstream of Pax2/8 are the transcription factor genes *Gata3* and *Lim1*, which are essential to later nephric duct elongation and guidance [16,17] (Fig. 1D). Furthermore, Lim1 acts to maintain the integrity of the nephric duct epithelium, as loss of *Lim1* after nephric duct establishment results in localized disruption of the ductal structure, potentially associated with a loss of epithelial cell characteristics, and an increase in apoptosis [16,18,19]. Lim1 has been shown to regulate the expression of several epithelium-specific genes, including *Cldn5* (claudin-5), *Pcdh19* (protocadherin-19) and *Npnt* (nephronectin) during urogenital system development, as well as normally suppressing the expression of the pro-apoptotic factor gene *Bmf* [20]. In this respect, proper establishment of adherence junction integrity may be important to maintain the nephric duct epithelium, as loss of *cdh17* (cadherin-17) in zebrafish is associated with degeneration of the posterior nephric duct and morphologically apparent duct blebbling and distortion [21]. Zebrafish *cdh17* is most closely related to the mammalian *CDH17*; however, these mutants show no obvious defects in early nephric duct development [22], suggesting that other cadherins are likely required in the nephric duct epithelium. One putative candidate is the closely related kidney-specific *Cdh16* (cadherin-16), which appears to be regulated through a Snail-HNF1b transcriptional cascade in kidney epithelia, although a nephric duct phenotype has yet to be described [23]. It is notable that no single mutants for adherens junction components show obvious nephric duct defects, strongly implying that, at least in mammalian systems, there is redundancy in the factors required for nephric duct epithelialisation and integrity. Intriguingly, loss of *Pax2* allows for the initial formation of a nephric duct due to redundancy with *Pax8*; however, maintenance of the nephric duct epithelium is compromised by a loss of epithelial cell polarity concomitant with an absence of adherens and tight junction proteins [24,25].

The establishment of a core Pax2/8, Gata3, Lim1 regulatory network may be of crucial importance to the continued survival and

and retinoic acid signalling, that result in directed cell migration of nephric duct tip cells and eventual fusion with the cloaca. (D) Nephric duct survival, differentiation, and migration programmes are coordinated by a core Pax2/8-Gata3-Lim1 regulatory network. Together with extrinsic signalling inputs (grey), such as retinoic acid or Fgfs, these intrinsic factors (green) activate effector molecules to execute specific cellular behaviours within the nephric duct. BMP, bone morphogenetic protein; ECM, extracellular matrix; FGF, fibroblast growth factor; GDNF, glial-cell derived neurotrophic factor; IM, intermediate mesoderm; LPM, lateral plate mesoderm; NC, notochord; ND, nephric duct; NT, neural tube; PM, paraxial mesoderm; RA, retinoic acid; RAR, retinoic acid receptor.

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differentiation of the nephric duct (Fig. 1D), as loss of upstream *Pax2/8* expression results in fulminant apoptosis, not only of the nephric duct but also surrounding mesenchyme, hinting at the presence of non-cell-autonomous survival factors regulated by *Pax* genes [1]. The onset of apoptosis in the nephric duct and adjacent tissue occurs nearly 24-h past the normal onset of *Pax2/8* expression, suggesting that *Pax* genes may indirectly regulate pro-survival or anti-apoptotic factors, likely through their control of the aforementioned gene regulatory network. The regulation of cell survival appears to be an important aspect of *Pax* gene function as increased cell death is also observed in $Pax2^{+/-}$ hypoplastic kidneys, a phenotype that can be rescued by concomitant *Bcl-2* expression [26]. In addition, loss of downstream *Lim1* results in eventual apoptosis of the nephric duct, as does loss of *Emx2*, again associated with loss of epithelial integrity [16,27]. Notably though, the extent and severity of degeneration upon loss of *Pax2/8* is more extensive than either loss of *Lim1* or *Emx2* alone.

Extrinsic trophic factors from the surrounding intermediate mesoderm also appear to support the survival and differentiation of the anterior nephric duct via a paracrine mechanism, potentially in cooperation with the cell-autonomous actions of Pax2/8 (Fig. 1D). A role for receptor-mediated MAPK signalling within the nephric duct comes from the remarkable observation that compound inactivation of *Fgfr1* and *Fgfr2* results in the absence of the entire duct at E10.5, whereas ablation of either single receptor does not affect the maintenance of the nephric duct [28]. Notably, although mesenchymally derived Fgf8 has been implicated in the maintenance of the anterior nephric duct, the requirement for *Fgf8* is less widespread than that of the receptors suggesting that other Fgfs may be involved [28]. Along these lines, ex vivo cultures have implicated Fgf 2, 7, and 9 as potential mitogenic factors for the nephric duct, as exogenous addition to culture medium results in significant thickening of the ductal diameter [29]. However, the relevance of these factors in vivo is still unclear.

4. Duct elongation and guidance systems

Once the nephric duct primordium is specified anteriorly and condenses to form an initial epithelial duct, it must extend caudally along a stereotypical path to the cloaca whereupon both tissues fuse to join the upper and lower urinary tracts. A variety of mechanisms exist to elongate epithelial tubes, many of which have been explored in amphibian and avian pronephric duct systems. Intriguingly, early descriptive studies highlighted differences in the mode of duct elongation between species, which have been postulated to adapt to species-specific developmental constraints by differential use of common genes, rather than by evolution of novel genetic mechanisms (reviewed in [30]). Regardless, commonalities have been described in the initial segregation of the pronephric primordium from a ridge of mesoderm lateral to the paraxial mesoderm. This short cord of cells becomes morphologically distinct from the surrounding mesoderm, as it loses many surrounding mesodermal contacts, while increasing long protrusion contacts to neighbouring nephric duct progenitor cells [31,32]. The pronephric duct rudiment elongates caudally along the ventral somite border, and appears to somewhat parallel the progression of somitogenesis [31–33], leading to the speculation that the factors driving initial elongation and guidance could either arise from the newly formed somites or the anterior–posterior differentiation of the embryo itself.

The mechanisms of elongation between model organisms differ mainly in the extent to which they are based on active cellular migration from the pronephric duct rudiment as opposed to cell segregation from the intermediate mesoderm. For example, frog embryos specify a larger area in which the nephric duct is segregated de novo from the intermediate mesoderm, and only involve active migration of duct cells in the most caudal regions, whereas zebrafish pronephric ducts appear to segregate along their entire length [30,31,34]. In contrast, axolotl, chick and mouse nephric ducts rely primarily on active cell migration and proliferation for elongation (Fig. 1B), as physical obstruction of the anterior nephric duct rudiment or cauterization of the leading tip cells results in the complete absence of posterior nephric ducts [35–40]. Interestingly, elevated rates of proliferation within the nephric duct are not definitively required to drive elongation, as axolotl nephric duct cells divide at a similar rate as the surrounding mesoderm [30]. Instead, convergence and extension movements appear to play a major role in axolotl nephric duct morphogenesis. A series of scanning electron microscopy and vital dye marking experiments revealed that the anterior nephric duct primordium narrows as it extends, a hallmark of convergence and extension, wherein thinning of the anterior region of the duct provides the physical force driving more posterior elongation/cell migration [30,35].

Conversely, in chick embryos there is no observable convergence of nephric duct tissue; instead, an increase in proliferation at the tip of the duct appears to supply the source of migratory nephric duct cells [40]. Recently, high resolution imaging of mouse and chick nephric ducts has demonstrated that the tip cells of the elongating duct are highly dynamic, sending out multiple cytoskeleton-based protrusions that may sense the environment and direct migration (Fig. 1B) [25,41,42]. Furthermore, in the chick system, RhoA-mediated tail retraction of the cells critically contribute to traction forces via actin-based stress fibre formation, as RhoA-inhibition results in nephric duct cells with aberrantly long tails that disrupt elongation [41]. Interestingly, alternative small GTPases may be required to regulate cell protrusions, as overexpression or inhibition of Rho signalling did not affect the cell behaviour of the tip cells [41]. Externally, the lamellipodia and filopodia-like extensions make contact with the underlying substratum containing collagen-fibrils and glycoproteins, such as fibronectin. Fibronectin is also enriched in the extracellular matrix of tip cells, and seems to play an essential role in the migration of avian nephric duct [43]. Ablation of fibronectin signalling through blocking antibodies results in stalling of the duct, likely through disrupted integrin signalling [43]. Additional support for the role of integrin signalling in nephric duct elongation comes from inhibitor experiments in axolotl embryos, which demonstrated a clear requirement for $\alpha 1\beta 6$-integrin signalling in migrating duct cells [44].

Surprisingly, tissue recombination experiments in amphibian and avian systems have demonstrated that the substratum upon which nephric duct cells migrate provides crucial guidance information, instead of acting as an inert surface, at least during early ductal elongation (Fig. 1B). In axolotl, the influence of the substratum appears to be developmentally modulated in parallel with paraxial mesoderm differentiation, perhaps shedding light on the mechanism by which nephric duct development is associated with somitogenesis [33]. Modulation of the substratum is achieved partially through the secretion of extracellular matrix components by the epidermis onto the migrating nephric duct path, which can be disrupted through functionally impeding laminin-integrin binding [44–46]. Notably, while embryo manipulations to reorient the epidermally derived extracellular matrix are sufficient to deflect nephric duct migration, blocking the cell-binding domain of laminin-1 prevents this redirection [44,46]. A similar experiment demonstrated that competitive inhibition of the laminin binding sites in avian extracellular matrix results in the misdirection of the migrating nephric duct in ovo [43]. Strikingly, the wandering nephric duct always migrated laterally over the lateral plate, suggesting that there are directional guidance cues emitted by the surrounding tissues that are normally disregarded by nephric

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duct cells in direct contact with a laminin-containing extracellular matrix [43].

The requirement for laminin in the extracellular matrix of migratory nephric duct cells appears to extend to mammalian systems as well, since deletion of the nidogen-binding module in the laminin-γ1 chain abrogates caudal extension of the duct, associated with local basement membrane disruptions of nephric duct tip cells [47]. The strict need for nidogens in this process is less clear, however, as neither single nor compound inactivation of *Nid1* and *Nid2* (nidogen-1 and -2) results in nephric duct dysgenesis [48]. It is possible that disruptions in the basement membrane of migrating tip cells inhibit communication mediated by extracellular matrix receptors, which may provide local guidance cues similar to the situation for chick and axolotl nephric duct elongation. In line with this, compound deletion of integrin receptors in mice results in disrupted elongation of the nephric duct, although the cellular mechanism behind this remains unclear [49].

Analysis of various mouse models substantiates the idea that nephric duct elongation proceeds through mechanisms similar to those observed in avian and amphibian embryos. Notably, embryo imaging with *Pax2-GFP* or *HoxB7-GFP* reporters also revealed cell protrusions characteristic of migrating cells at the tip of mammalian nephric ducts prior to fusion with the cloaca (Fig. 1C) [25,42]. It is interesting that, at the stages imaged, only the few tip cells appear to extend protrusions, which is a characteristic of leading cells in collective cell migration. Furthermore, the cellular extensions are apparently stabilized caudally, in the direction of migration, strongly suggesting guidance by an attractive cue (Fig. 1C).

Control of nephric duct guidance is achieved in part by the transcription factor Gata3 (Fig. 1D), as null embryos show an impaired sensitivity to guidance cues, despite normal expression of integrins and extracellular matrix components [17]. A subset of *Gata3* mutant nephric ducts are indeed attracted to the surface ectoderm, revealing the presence of previously unsuspected guidance cues acting at an early stage during the elongation process that require *Gata3* for interpretation [17]. Interestingly, conditional inactivation that bypasses the early requirement for *Gata3* results in a nephric duct guidance and cloaca connection defect [42].

Together with retinoic acid signalling, Gata3 is required for *Ret* expression in the nephric duct [42,50,51]. Accordingly, mouse embryos deficient for *Ret* and or the retinoic acid synthesis enzyme *Raldh2* also fail to properly insert the nephric duct in the cloaca [42]. Turning of the migrating duct towards the midline and fusion with the cloaca is accompanied by active sensing by the tip cells, which extend multiple thick protrusions that are strongly impaired in all three mutant embryos (Fig. 1C) [42]. In addition, the inactivation of the *Ret* ligand *Glial-cell derived neurotrophic factor* (*Gdnf*) results in a connection defect between the upper and lower urinary tracts, suggesting a similar nephric duct-cloaca connection phenotype [52]. Hence, Gdnf may also be part of the guidance system driving duct elongation and fusion (Fig. 1D).

Activation of Ret by Gdnf results in the phosphorylation of critical docking residues for adaptors involved in either PLCγ or PI3K/MAPK pathway activation, which are known to regulate cellular processes including cytoskeletal rearrangement and migration. Interestingly, a homozygous *Y1015F Ret* mutation, deficient in PLCγ-activation, did not affect nephric duct insertion into the cloaca [53,54]. In contrast, *Y1062F Ret* mutants deficient in PI3K/MAPK activation exhibit urogenital system phenotypes compatible with defects in nephric duct elongation [53,54]. In response to chemotactic agents, PI3K is often activated at the leading edge of migrating cells to regulate protrusion formation, whereas the lipid phosphatase PTEN, which inactivates PI3K-signalling, modulates tail retraction and cell body movement [55]. Furthermore, activation of the MAPK pathway has been shown to regulate proliferation of the caudal nephric duct, thus providing potential insight into the mechanisms by which Ret signalling promotes late elongation and path finding activity of the nephric duct [53]. Together these results suggest that the Gdnf-Ret signaling system is an important player in the late phase of nephric duct elongation and connection to the cloaca. Of interest, the Gdnf-Ret duct elongation system seems to be evolutionarily conserved, as axolotl nephric ducts are also guided by a gradient of Gdnf expression that activates its cognate receptor complex GFRα1-Ret tyrosine kinase in the nephric duct [56]. In vitro studies with kidney epithelial cells have additionally demonstrated that they form lamelipodia and filopodia, rearrange their actin cytoskeleton, and migrate directionally in response to activation of the GDNF-Ret signalling pathway [57].

Nephric duct elongation, guidance and fusion to the cloaca represent an impressive example of morphogenetic orchestration at the cellular level. The failure or improper timing of any of these cell behaviours leads to a range of developmental defects including complete kidney and urinary tract agenesis, urinary and genital tract fusion and vesicoureteral reflux [18,42,51], that are collectively part of the Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) developmental disease group. This medical condition is characterized by a large spectrum of developmental defects often observed within families [58]. The phenotypical variability observed is likely to stem in part from defective coordination of morphogenetic cell behaviours. Additionally, some plasticity in the system is expected from imperfect rescue, most notably by bladder growth that is predicted to re-establish defective upper and lower urinary tract connections.

5. Epithelial cell plasticity

During urogenital system development, the intermediate mesoderm is specified along the anterior–posterior axis to give rise to different mesenchymal populations of cells with distinct inductive capabilities. Of particular interest, the intermediate mesoderm adjacent to the hindlimb is specified such that it can induce a diverticulum from the nephric duct to invade and undergo iterative branching, eventually giving rise to the metanephric kidney (reviewed in [3] and by Blake and Rosenblum, this issue). This anteroposterior patterning of the intermediate mesoderm appears to be defined by a specific *Hox* gene code, similar to that described for the specification of neural crest precursors [59]. The *Hox11* gene family, in particular, demarcates the metanephric mesenchyme adjacent to the hindlimb by regulating expression of crucial factors for its differentiation, including *Gdnf* and *Six2* [60,61]. Proper positioning of the metanephric mesenchyme is further controlled by an additional layer of transcription factor genes, including *Foxc1/2*, that delineate anterior boundaries by repressing expression of *Gdnf* (see review by Blake and Rosenblum in this issue). The responsiveness of the nephric duct to inductive signals is also regulated along the anteroposterior axis by secreted factors, thus ensuring ureteric budding is restricted to a single site [61].

Setting up the ability to respond to inductive signals within the nephric duct seems to be an active process, mediated in large part by heterogenous levels of Ret signalling that likely result in differential activation of the MAPK pathway effectors (Fig. 2A). Chimeric nephric duct organ culture using cells deficient for *Ret* or for its intrinsic modulator *Spry* has shown the presence of concerted movements of individual duct cells to sort highly Ret-responsive cells to the dorsal side, where the ureteric bud will arise [62]. It has been postulated that this could serve to focus the nephric duct response to generate a discrete ureteric bud in the face of the broad swathe of *Gdnf* expression that occurs in the ureteric bud region (Fig. 2A) [62]. If true, such a mechanism provides an elegant double-layered system wherein ureteric bud induction is

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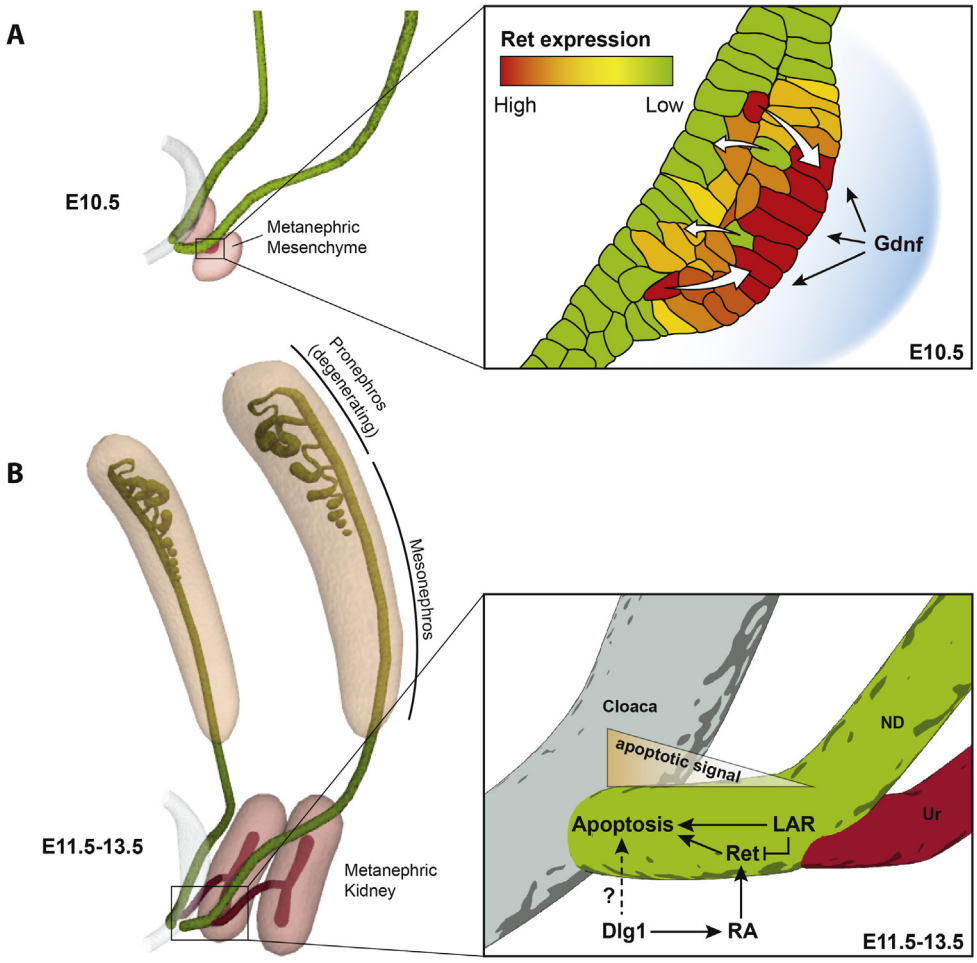

Fig. 2. Interactions between mesenchymal and nephric duct epithelial tissues drive cell sorting to form the ureteric bud, and programmed cell death during ureter maturation. (A) Ureteric bud evagination involves Ret tyrosine kinase-dependent cell sorting (white arrows) to assemble the highest Ret-responsive cells towards Gdnf secreted from the metanephric mesenchyme. (B) A graduated apoptotic signal drives elimination of the common nephric duct. Apoptotic cell death involves Dlg1 and retinoic acid signalling from the mesenchymal compartment, which appear to act through Ret within the common nephric duct. Apoptosis additionally requires the expression of LAR-family receptor protein tyrosine phosphatases in the common nephric duct, which act partially by downregulating Ret pro-survival signalling. ND, nephric duct; Ur, ureter.

controlled extrinsically by the metanephric mesenchyme, and cell-autonomously within the nephric duct itself such that only the most responsive cells are positively selected to contribute to ureteric bud formation.

Contrary to expectation, cell sorting occurs without delamination of nephric duct cells from the epithelium [62], although it likely requires epithelial cell junction remodelling, resulting in a nephric duct with epithelial plasticity. In line with this, nephric duct formation and elongation progresses with cells that are more loosely connected [33], and is followed by a wave of differentiation that establishes E-cadherin dependent connections (KS personal observations). Similarly, adjacent to the metanephric mesenchyme, the nephric duct transitions from a simple, single layered epithelium to a pseudostratified epithelium concomitant with the localized swelling that generates the ureteric bud [62]. This localized swelling involves an increase in cell proliferation, potentially to generate the cells needed for pseudostratification of the epithelium and eventual evagination to form the ureteric bud [63]. It is thought that signals from the mesenchyme surrounding the nephric duct may be responsible for initiating the epithelial transition, as mutants for the intermediate mesoderm marker *Osr1* remain as a simple epithelial duct [62,64,65]. Oddly enough given the dominant role for the Ret-Gdnf axis in ureteric budding, Ret-negative ducts become pseudostratified normally, suggesting that alternative signalling pathways control the temporal and spatial aspects of epithelial transitioning within the nephric duct [62]. Thus, dual mechanistic cell behaviours exist within the nephric duct to correctly form the ureteric bud; one that entails Ret-dependent cell sorting, and one involving Ret-independent epithelial reorganization. Of interest, mosaic deletion of *Gata3* in the nephric duct also led to ureteric bud cell sorting, which likely results from *Ret* downregulation in *Gata3*-deficient cells [50]. In parallel with this, an upregulation in duct differentiation occurs, similar to that seen upon loss of β-catenin (*Ctnnb1*) in the nephric duct [50,66]. In fact, further examination of the mutant embryos places β-catenin genetically upstream of *Gata3* during caudal nephric duct development [50] (Fig. 1D). It seems possible then that *Gata3* may regulate both ureteric bud cell sorting through regulation of Ret expression, and epithelial swelling and differentiation through an unknown repressive mechanism.

6. Programmed cell death and ureter maturation

The final step in early nephric duct development involves the separation of the genital and urinary tracts. Following ureter budding from the nephric duct, the urinary tract must undergo remodelling to separate the ureter from the nephric duct and insert it into the primitive bladder, leaving the nephric duct connected to the future urethra (reviewed in [58,67]). Detailed investigation of mouse models and urogenital system cultures has shown that

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this normally proceeds in two steps; first, the segment of nephric duct between the ureter branch point and the cloaca (the common nephric duct) is eliminated by apoptosis, bringing the ureter into the proximity of the urogenital sinus [4,5]. Following this, the distal portion of the ureter is also removed by apoptosis, and a new ureterovesical junction is established [4,5].

Initial elimination of the common nephric duct segment occurs through a gradient of apoptosis that is most intense in the caudal region and lessens towards the ureter branch point, but is specific to the ductal epithelium (Fig. 2B) [5]. Within the nephric duct, apoptosis requires the expression of the LAR-family receptor protein tyrosine phosphatases, which act in part by downregulating pro-survival Ret tyrosine kinase signalling [5]. Unfortunately, the balance of signalling is not so straightforward, as Ret also appears to be required for apoptosis-mediated ureter remodelling [4,68]. Interestingly, elimination of retinoic acid signalling by ablation of either retinoic acid receptor genes *RARa/RARb2* or *retinaldehyde dehydrogenase 2* (*Raldh2*) in the mesenchyme surrounding the nephric duct results in the absence of distal ureter apoptosis, concomitant with downregulation of *Ret* expression in the nephric duct [4,68]. Forced expression of Ret in nephric duct derivatives is able to rescue ureter remodelling, strongly implicating mesenchymally derived retinoic acid in propagating apoptotic signalling within the nephric duct [4]. However, interpretation of these studies is complicated by the fact that common nephric duct and subsequent distal ureter apoptosis requires contact with the urogenital sinus, which is defective in at least a subset of retinoic acid and *Ret* mutant animals (see above).

Further insight into the function of the mesenchyme in regulating nephric duct apoptosis comes from conditional deletion of *Dlg1* in the peri-ductal mesenchyme, which results in downregulation of retinoic acid signalling components and subsequent decreased *Ret* expression in the nephric duct [6,69]. Notably, elimination of mesenchymal *Dlg1* was sufficient to completely eliminate common nephric duct apoptosis [6], suggesting that propagation of apoptotic signals requires a mesenchymal component and is not solely emanating from the cloacal epithelium as suggested by the massive apoptotic rate at the nephric duct-cloaca junction during the maturation process (Fig. 2B).

From a mechanistic standpoint, appropriate elimination of the common nephric duct and subsequent ureter remodelling is crucial for the proper functioning of the urinary tract. An absence or delay in ureter remodelling results in uterovesical junction obstruction (reviewed in [58]), which, in severe cases is associated with massive renal damage and end stage renal disease. Conversely, it seems likely that an over-activation of apoptosis within the nephric duct would result in an acceleration of ureter descent, and subsequent lateral insertion in the bladder, potentially resulting in faulty ureterovesical junctions and urine reflux. Often such situations are ascribed to caudal budding of the ureter, which can be viewed as equivalent to reducing the amount of common nephric duct to be eliminated, thus accelerating the entire process. However, such a prediction has yet to be functionally demonstrated.

7. Conclusion

Together with early descriptive studies and tissue recombination experiments, genetic manipulation of model organisms has revealed many cellular behaviours that occur during the early steps of urogenital system morphogenesis, although a clear understanding of the molecular networks that drive these behaviours lags behind. It is equally clear that the coordinated signalling between the multiple tissue compartments and cell types that comprise the urogenital system is critical for the spatial and temporal organization of urogenital development. Importantly, the mechanisms that underlie many childhood urinary tract diseases result from aberrant cell behaviours during early nephric duct morphogenesis, and so it is to be hoped that, as the detailed understanding of these behaviours and the underlying genetic networks grow, it may lead to better and improved interventions in congenital urogenital malformations.

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